Effects of Ultraviolet B Light on Cutaneous Immune Responses of Humans with Deeply Pigmented Skin

Martin Vermeer, George J. Schmieder, Takeshi Yoshikawa, Jan-Willem van den Berg, Michael S. Metzman, J. Richard Taylor, and J. Wayne Streilein

Departments of Microbiology and Immunology (MV, TY, J-W vdB, JWS) and of Dermatology and Cutaneous Surgery (GJS, JRT), University of Miami School of Medicine, and the Veterans Administration Hospital of Miami (JRT), Miami, Florida, U.S.A.

The incidence of skin cancers of the basal and squamous cell types is extremely low among genetically black-skinned human beings, whereas these types of skin cancers are common among Caucasians, especially those who live in geographic areas of high sun exposure. Ultraviolet B light (UVB) is thought to be the primary oncogenic agent in sunlight. We have recently demonstrated that acute, low-dose exposure of Caucasian skin to UVB impairs the induction of contact hypersensitivity to dinitrochlorobenzene (DNCB) in approximately 40% of normal individuals. Importantly, this trait — termed UVB susceptibility — was found to be a characteristic of virtually 100% of patients with a history of biopsy-proved skin cancer, implying that UVB susceptibility may be a risk factor for this disease. Because melanin pigment is thought to be protective of some of the deleterious effects of UVB radiation, we have examined the capacity of a low-dose regimen of UVB to alter induction of contact hypersensitivity in individuals with genetically melanized or heavily tanned skin. Our results indicate that UVB radiation depletes heavily pigmented skin of Langerhans cells, just as it does in Caucasian skin. Moreover, UVB-susceptibility exists as a polymorphic trait in individuals with genetically determined black skin, as well as in individuals with heavily tanned skin, and the incidence of this trait is similar to that found among normal Caucasian subjects. Thus, melanin does not appear to protect against the deleterious effects of an acute, low-dose regimen of UVB on induction of cutaneous immunity, and the UVB susceptibility trait is equally well-represented in both black- and Caucasian-skinned individuals. We conclude that although UVB susceptibility may function as a risk factor for skin cancer in Caucasians, it does not function similarly in black-skinned human beings, probably because melanin effectively protects against the mutagenic properties of UVB radiation. *J Invest Dermatol 97:729–734, 1991*

Chronic exposure to sunlight carries an increased risk of development of squamous and basal cell cancers in human beings. The epidemiologic evidence in support of this statement is particularly compelling in certain geographic regions of the earth, such as Australia, southwestern United States, and south Florida, where daily sunlight is very high [1–3]. There is a general consensus that the component of sunlight that is primarily responsible for inducing skin cancer is within the ultraviolet B spectrum (290–320 nm) [4–6]. By the same token, UVB radiance within sunlight also appears to be responsible for induction of melanization (tanning) [7], and it is believed that tanned skin is relatively protected from the oncogenic properties of sunlight. The fact that squamous and basal cell carcinomas are rare in individuals with genetically melanized skin is taken as further evidence that melanin pigment is a photoprotector [8].

UVB is thought to promote skin cancer through several distinctly different, but complementary, mechanisms. First, UVB disrupts the molecular structure of certain amino acids, nucleic acids, and lipids [9,10]. The culmination of these reactions is the creation of mutations within the DNA of irradiated cells. It is believed that some of these mutations can result in unregulated growth and malignant degeneration of the target cells [4–6]. Second, work in experimental animals has demonstrated that UVB radiation of skin can be damaging to the immune system [11,12], and it has been reasoned that UVB-induced injury to the surveillance properties of the immune system decreases the likelihood that malignant clones of cells harboring UVB-induced mutations can be eliminated before they develop into full-fledged cancers.

We have recently reported that acute, low-dose UVB irradiation of untanned skin impairs the induction of contact hypersensitivity to dinitrochlorobenzene (DNCB) in approximately 35–40% of normal, adult Caucasian human beings [13]. These results indicate that the human species displays polymorphisms that govern susceptibility to the deleterious effects of this dosage of UVB. We have termed those individuals who fail to develop contact hypersensitivity when DNCB is painted on UVB-exposed skin as "UVB susceptible;" their counterparts, who develop contact hypersensitivity even when hapten is painted on UVB-irradiated skin, are termed "UVB resistant." In our recent report, we also demonstrated that low-dose UVB light impaired the induction of contact hypersensitivity in patients with biopsy-proved skin cancer. Moreover, the incidence of UVB-susceptibility among these patients was found to be 92%. Consequently, we have proposed that susceptibility to the deleterious effects of UVB on contact hypersensitivity in man may be an important risk factor for the development of skin cancers of this type.

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Reprint requests to: Dr. J. Wayne Streilein, Department of Microbiology and Immunology (R-138), University of Miami School of Medicine, P.O. Box 016960, Miami, FL 33101.

Abbreviations:
DNCB: dinitrochlorobenzene
PAR: primary allergic reaction
TNF_α: tumor necrosis factor-alpha
UVB: ultraviolet B light

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Table I. Effect of Ultraviolet B Light on Langerhans Cell Densities in Epidermis of Deeply Pigmented Human Skin

Patient Number	Sex	Age	Skin Color	Langerhans Cells (mm^2) Unirrad	Irrad
110	F	25	B-2	466	<20
111	F	39	B-3	466	<20
112	F	45	B-3	484	<20
113	F	24	B-4	503	<20
114	F	45	B-4	446	<20
115	F	40	B-5	484	<20

The infrequency of squamous and basal cell cancers in individuals with black skin, compared to persons with Caucasian skin, made us wonder whether melanization protects skin against the deleterious effects of UVB on the immune system. To examine this issue, we have evaluated the UVB susceptibility of Caucasian subjects with heavily tanned skin and of individuals with genetically dictated, deeply pigmented skin. Our results indicate that UVB susceptibility is a polymorphic trait that is expressed in black-skinned individuals, and that facultative melanization of skin does not prevent UVB radiation in the doses employed from impairing the induction of contact hypersensitivity.

MATERIALS AND METHODS

Subjects Normal human volunteers were selected on the basis of having deeply pigmented skin due to their genetic constitution. All volunteers had sun-reactive skin of type V and VI, as classified by Fitzpatrick [14]. Thirty black-skinned individuals ranged in age from 19–52 years; two men and 26 women were of African ancestry, whereas two women, 19 and 31 years old, were of Indian (Asiatic) background. Characteristics of these volunteers are presented in Tables I and II.

Intensity of melanization was assessed by matching one of five Dermablend Cover Creams (Dermablend Corrective Cosmetics, Farmingdale, NY) to the buttock skin of each individual. Dermablend Cosmetics has developed eight different shades to conceal imperfections of the skin, varying from vitiligo to birthmarks. Each cover cream is classified with a number ranging from 0 to 5, with 0 being the lightest shade, and 5 the darkest. In general, skin colors matching Dermablend Chroma 2 and 3 correlated with sun-reactive skin type V, and skin colors matching Dermablend Chroma 4 and 5 correlated with sun-reactive skin type VI. The following scoring system was used to classify our black-skinned patients:

Cosmetics Designation	Our Scale
Chroma 1	B-1
Chroma 2	B-2
Chroma 3	B-3
Chroma 4	B-4
Chroma 5	B-5

Each cream was placed in numerical order on a plain white 5 × 8 inch index card, the cards were placed adjacent to buttock skin, and the best match was determined by direct inspection.

Four Caucasian male volunteers, ranging in age from 21–28 years, who worked as lifeguards for several months and whose skins were heavily tanned, were also used. The volunteers were all free of any known disease and none was taking any medications at the time of these studies. All participants gave informed consent.

Ultraviolet B Irradiation The method used for delivering UVB light to buttock skin was described previously [15]. Briefly, a high-pressure mercury lamp (Dermalight System, Dr. Honle, Munich, West Germany), emitting UV light with peaks at 300 and 310 nm, was used. An area of buttock skin was chosen as the irradiated site in all cases and the area of irradiation comprised a circle of 2 cm diameter. Under these conditions, the irradiance in the UVB range (290–320 nm) at skin level was 1.6 mW/cm^2. All subjects were exposed to 144 mJ/cm^2 per day for four consecutive days.

Enumeration of Epidermal Langerhans Cells UVB-irradiated skin from each of six subjects was biopsied within 1 h after the final (fourth) UVB exposure. The center of the irradiated site was anesthetized with 1% lidocaine HCl and a 3-mm punch biopsy was taken. Standard procedures for immunofluorescence studies on epidermal sheets were followed with minor modifications. Briefly, excess fat was trimmed from biopsy specimens and each sample was placed in EDTA for 3 h at 37°C. Epidermal sheets were separated from dermis and fixed in acetone for 15 min. The specimens were then washed in phosphate-buffered saline (PBS) and incubated 16 h with 1 : 10 diluted anti-Leu 6 (CD1) antibodies, which was obtained from Becton Dickinson Immunocytometry Systems (Mountain View, CA). The epidermal sheets were washed in PBS and exposed to 1 : 20 diluted fluoresceinated goat anti-mouse IgG (Becton Dickinson, Mountain View, CA) for 1 h. The sheets were then washed and mounted in 9 : 1 glycerol : PBS. The density of positively staining cells was assessed using an Olympus BH-2 immunofluorescence microscope equipped with a micrometer with a 1-cm^2 reticule divided into 100 squares. Ten different areas of each specimen were counted and an average number of positive cells calculated. Biopsies of non-irradiated buttock skin from each of the six subjects was also taken and the density of Langerhans cells was assessed.

Antigen Dinitrochlorobenzene (DNCB) was obtained from Sigma Chemical Co. (St. Louis, MO) and used as sensitizing agent. For sensitization, 20 mg of DNCB was diluted to 1.0 ml in acetone (2000 $\mu g/100\ \mu l$). For elicitation this solution was further diluted with acetone to reach a final concentration of 50 $\mu g/100\ \mu l$.

Sensitization and Elicitation of Contact Hypersensitivity As described previously [13], 2000 $\mu g/100\ \mu l$ DNCB in acetone, which is known to be universally sensitizing dose in normal Caucasians [16], was applied carefully to irradiated buttock skin within 1 h of the fourth UVB treatment. The diameter of the application site was 1.8 cm, and care was taken to ensure that no hapten spilled

Table II. Clinical Features of Volunteers with Deeply Pigmented Skin[a]

Patient Number	Sex	Age	Skin Color
38	F	25	B-4
44	F	40	B-4
45	F	48	B-3
48	M	52	B-4
65	F	32	B-3
69	F	49	B-4
70	F	25	B-5
71	F	31	I-3
72	F	21	B-4
81	F	35	B-4
82	F	19	B-4
85	F	26	B-4
86	F	36	B-3
87	F	40	B-3
97	M	28	B-2
101	F	29	B-4
104	F	19	I-3
105	F	50	B-4
98[b]	F	26	B-3
102[b]	F	41	B-2
103[b]	F	31	B-4
107[b]	F	29	B-2
108[b]	F	34	B-4
109[b]	F	36	B-3

[a] B, African origins; I, Indian (Asiatic) origins.
[b] Positive controls (no UVB).

beyond the margin of the irradiated site. The painted area was occluded with paper tape for 24 h. Primary allergic reactions (PAR) were detectable as early as 6 d to as late as 14 d following hapten application. Elicitation of contact hypersensitivity was accomplished by painting 50 μg DNCB in 100 μl acetone on the ventral surface of the forearm. Cutaneous responses were assessed clinically 2, 4, and 7 d thereafter, according to the scoring method described below.

Clinical Scoring of Cutaneous Inflammatory Responses Cutaneous inflammation was elicited by ultraviolet B irradiation (phototoxic reaction), assayed immediately after the fourth dose of UVB radiation; by DNCB (toxicity reaction), assayed 24 h after application of sensitizing dose of hapten; during the PAR, assessed between 6 and 14 d following sensitization; and at the challenge site, assessed 2, 4, and 7 d following forearm challenge with DNCB. The following scoring system was used: 1+, erythema only; 2+, erythema plus edema; 3+, erythema, edema, plus vesicle formation; 4+, blister formation and necrosis of the epidermal surface.

Experimental Plan Eighteen black-skinned and four deeply tanned Caucasian subjects participated in the following experimental protocol, which has been described previously [13]: days 3, 2, 1, and 0 — UVB irradiation of buttock skin. Day 0 — assess phototoxic response; apply 2000 μg DNCB to irradiated skin. Day 1 — assess DNCB toxic response. Day 6, 10, 12, 14 — assess Primary Allergic Reactions, if any. Day 30 — apply 50 μg DNCB in acetone to forearm skin. Days 32, 34, 37 — assess for contact hypersensitivity response. In general there was good correspondence between the challenge reaction and the PAR. However, the arbitrary decision was made to consider the response at the challenge site only when designating a subject as UVB-susceptible or not. Some of the UVB-susceptible individuals were subjected to a second round of sensitization as a means of assessing immunologic tolerance. In these individuals, the following additional protocol was followed: day 51 — apply 2000 μg DNCB to buttock skin not previously exposed to UVB or painted with hapten. Day 52 — assess for DNCB toxicity. Days 57, 61, 62, 65 — assess development of PAR. Day 81 — apply 50 μg DNCB to forearm skin, avoiding site of previous challenge. Days 83, 85, 88 — assess for contact hypersensitivity response. As positive controls, six black-skinned subjects received 2000 μg DNCB to unirradiated buttock skin on day 0 and were challenged with 50 μg DNCB to forearm skin 30 d later in order to investigate whether 2000 μg DNCB was a universally sensitizing dose in black-skinned individuals.

RESULTS

Effects of UVB on Epidermal Langerhans Cells in Deeply Melanized Skin Irradiation of human skin with UVB reaches all epidermal cells, including Langerhans cells [17,18]. In mice, hamsters, and humans exposure of skin to low doses of UVB (400–600 J/m²) causes a sharp reduction in cells bearing surface markers typical of Langerhans cells [19,20]. We and others have suggested that the damaging effects of UVB on epidermal Langerhans cells may be important in the phenomenon whereby UVB light impairs the induction of contact hypersensitivity [21,22]. In human beings with Caucasian skin, we have shown that four daily exposures to 144 mJ/cm² reduces the number of $CD1^+$, $HLA\text{-}DR^+$ cells in the epidermis to virtually non-detectable amounts [14]. As melanin can absorb energy within the UVB range, we examined the effects of low-dose UVB radiation on Langerhans cells in melanized skin. Six healthy, adult black female volunteers were selected for this study. The intensity of endogenous melanization was assessed by matching one of five Dermablend Cover Creams to buttock skin sites. As indicated in Table I, the intensity of melanization at buttock skin sites to be irradiated in these individuals ranged from B-2 to B-5 (see *Materials and Methods*). On four successive days, a 2-cm diameter area of buttock skin received 144 mJ/cm² of UVB radiation. Within 1 h of the last exposure, skin biopsies were taken from the irradiated site, and from a distant, unirradiated site that displayed the same degree of melanization. The number and morphology of Langerhans cells was assessed by fluorescent microscopy of epidermal sheets, stained with anti-Leu6 (CD1) antibodies. The results, displayed in Table I, indicate that virtually no normal-appearing, $CD1^+$ cells could be identified in the UVB-exposed epidermis of any of these volunteers. Rarely, we observed rounded, positively stained images, but we could not be certain that these structures represented intact cells. The profound reduction in identifiable Langerhans cells we found in the skin of these black volunteers strongly resembled the depletion of normal-appearing Langerhans cells observed in Caucasian skin similarly treated with UVB [14]. We conclude that black skin, even that which is heavily melanized, responds to UVB radiation in a manner similar to Caucasian skin in that epidermal Langerhans cells are profoundly altered such that virtually no normal-appearing cells remain at the site. Thus, melanin does not appear to protect Langerhans cells from the damaging effects of UVB as used in this treatment protocol.

Effects of UVB on Induction of Contact Hypersensitivity in Black-Skinned Individuals Because melanin failed to provide photoprotection for Langerhans cells, we next examined the capacity of melanin to protect the skin from the deleterious effects of UVB on induction of contact hypersensitivity. A group of (18) black-skinned individuals served as volunteers for this study. All but two were of African origins; two originated from the Indian subcontinent. Sex, age, and skin color of these individuals are recorded in Table II. All subjects were free of any disease at the time of study, and none were taking medications. Each member of the group received four consecutive daily doses of 144 mJ/cm² UVB to buttock skin. Immediately after the last exposure, the epidermis of the irradiated site was painted with 2000 μg DNCB. The site was observed subsequently for evidence of a primary allergic reaction (PAR). Thirty days thereafter, the volar surface of the forearm was challenged with 50 μg DNCB and the site examined for development of contact hypersensitivity. Unlike our experience with the skin of Caucasian subjects, phototoxicity was infrequent and of low magnitude at UVB-exposed sites in black volunteers. Only five of these individuals displayed evidence of phototoxicity (data not shown). By contrast, the vast majority of white-skinned individuals experience phototoxic responses to this regimen of UVB [13]. Between 7 and 14 d post-epicutaneous application of hapten, 12 volunteers developed primary allergic reactions at the site (67%). The data are summarized in Fig 1. Moreover, upon forearm challenge at 30 d post-irradiation, only nine of 18 individuals (50%) displayed contact hypersensitivity, and the positive responses were in the 2–3+ range of intensity. Based on our previous studies in Caucasian individuals, we would designate the nine subjects (47%) who failed to develop contact hypersensitivity as "UVB susceptible." Thus, melanin pigment does not prevent UVB light from impairing the induction of contact hypersensitivity in a significant proportion of black-skinned individuals. To emphasize this point, we have grouped the results from these individuals according to intensity of cutaneous melanin deposition (see Table III). It is apparent that even in individuals with the most heavily pigmented skin (skin colors B4 and B5), UVB light is still able to reveal the UVB-susceptibility trait. Thus, melanin protects neither Langerhans cells from the damaging effects of UVB, nor the cutaneous immune system of certain individuals from UVB-induced impairment of contact hypersensitivity.

Effects of UVB on Induction of Contact Hypersensitivity in Individuals with Heavily Tanned Skin Although genetically determined melanization appeared not to protect against the cutaneous immune effects of UVB, the possibility exists that epidermal melanization resulting from prolonged sun exposure, i.e., tanning, might be protective. To examine this point, four adult male Caucasian volunteers participated in a study similar to that described above. All had worked as lifeguards on south Florida beaches for several months prior to the study. The clinical features of these individuals are summarized in Table IV. UVB was administered to a 2-cm diameter area on the back, an area that was above the trunk line and that was heavily tanned. As in the black-skinned individu-

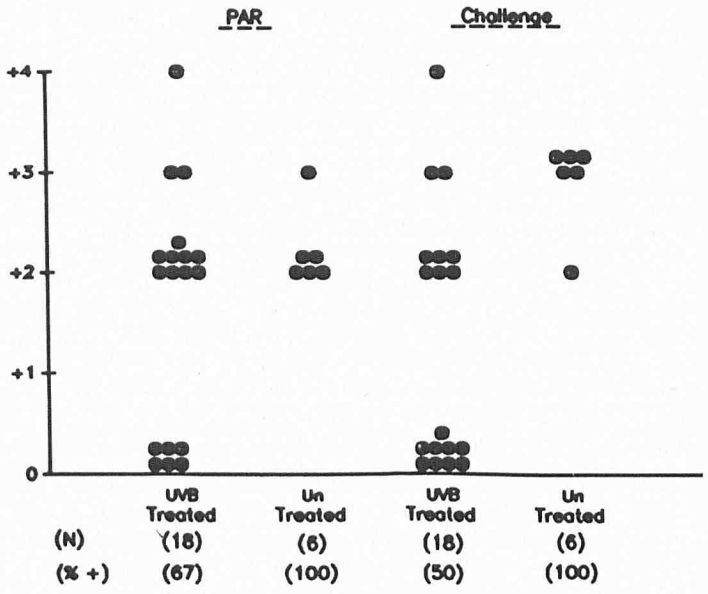

Figure 1. Effect of UVB on induction of contact hypersensitivity in normal black-skinned human beings. Clinical scores, ranging from 0 to 4+, for each individual are represented by *dots* on the figure. PAR refers to incidence of primary allergic reactions. Challenge refers to intensity of responses at forearm elicitation sites that received 50 μg DNCB 30 d after primary exposure to the hapten. UVB-treated individuals were exposed to UVB radiation protocol (as described in *Materials and Methods*) prior to skin painting with 2000 μg DNCB. Untreated individuals received 2000 μg DNCB without prior exposure to UVB radiation. (N), number of individuals displaying a clinical score of 1+ or greater.

als, phototoxicity was not observed at the irradiation sites in these volunteers. After 2000 μg DNCB was applied to the site, primary allergic reactions developed in all volunteers. However, when their forearms were challenged with 50 μg DNCB at 30 d, only two subjects displayed contact hypersensitivity. By the criteria we have employed previously [13], subjects 66 and 67 are designated as UVB-susceptible. Although this sample is small, we believe that the finding that two of four heavily tanned subjects are UVB susceptible is significant because 2000 μg DNCB is a universally sensitizing dose [15]. Thus, deeply pigmented skin, whether resulting from constitutive or facultative melanization, is not impervious to the deleterious effects of UVB on cutaneous immunity.

Table III. Relationship Between Intensity of Pigment and UVB-Susceptibility[a]

Patient Number	Skin Color	UVB Phenotype
97	B-2	Resistant
45	B-3	Susceptible
65	B-3	Resistant
71	I-3	Resistant
86	B-3	Resistant
87	B-3	Resistant
104	I-3	Resistant
38	B-4	Resistant
44	B-4	Susceptible
48	B-4	Susceptible
69	B-4	Susceptible
81	B-4	Susceptible
72	B-4	Susceptible
82	B-4	Resistant
85	B-4	Resistant
101	B-4	Susceptible
105	B-4	Susceptible
70	B-5	Resistant

[a] Subjects have been arranged according to degree of melanization, with lightest pigment first and darkest pigment last.

Induction of Hapten-Specific Tolerance Following Exposure to DNCB Through UVB-Treated, Melanized Skin We have previously reported that approximately 50% of skin cancer patients respond to DNCB painted on UVB-exposed skin by becoming immunologically unresponsive [13]. That is, hapten painted on UVB-treated skin actually induces tolerance in these patients. By contrast, UVB susceptible, normal Caucasian subjects do not appear to develop hapten-specific tolerance. To extend our understanding of this important phenomenon, we studied UVB-susceptible black-skinned, as well as heavily tanned Caucasian, subjects for the development of tolerance. Within 30 and 60 d of the initial forearm challenge with DNCB, black individuals designated as UVB susceptible received 2000 μg DNCB on buttock skin at sites that had not previously been irradiated or painted with hapten. These sites were observed for primary allergic reactions. The forearms of these subjects were challenged for contact hypersensitivity 30 d later. The results are presented in Table V. With *one exception,* every subject developed a primary allergic reaction, and went on to display contact hypersensitivity at a forearm challenge site. These results indicate that hapten-specific tolerance was not induced in UVB-susceptible individuals by an initial experience with hapten painted on UVB-exposed skin. However, one individual, patient 44, developed no primary allergic reaction, nor did she develop contact hypersensitivity at the challenge site during these re-immunization studies. She represents the first normal (non-skin cancer) individual we have studied in whom hapten painted on UVB-exposed skin induced immunologic tolerance. We believe that the best explanation for the unresponsiveness in this subject is that tolerance has been induced, because we have subsequently demonstrated that she was fully capable of developing contact hypersensitivity when immunized through normal skin with an unrelated hapten, diphencyprone (data not shown).

DISCUSSION

Although epidemiologic evidence indicates that black-skinned people have a low risk of developing skin cancer, even in areas of the world where indigenous sun exposure is very high, our experiments reveal that melanization does not appear to protect the epidermis from some of the deleterious effects of ultraviolet B radiation. In our studies, epidermal Langerhans cells were found to be profoundly altered (perhaps even destroyed) when acute, low doses of UVB radiation were delivered to heavily pigmented skin of black individuals, of either African or Asiatic origins. In addition, a high proportion of normal black-skinned adults proved to be UVB-susceptible, as defined by the inability to develop contact hypersensitivity when DNCB was applied to UVB-exposed skin. Moreover, Caucasian subjects with deep, sun-induced tans were able to display the UVB-susceptibility trait, even though neither they nor most of the black-skinned individuals developed phototoxicity at the UVB-irradiated site.

Our results confirm and extend the work of others who have articulated the claim that melanization of skin does not protect against some of the damaging features of sunlight [23-25]. Morrison in particular has questioned whether the primary function of melanin is photoprotection. In his view, the principal data supporting the photoprotection hypothesis are a) that albinos living in the tropics die of skin cancer in early adult life, and b) that transplanted and immunosuppressed Europeans have a high incidence of skin cancer in tropical and subtropical climates. He proposes that melanization of skin is evolutionarily important, not with regard to photoprotection, but as a camouflage and/or as a heat absorber. Our data also fail to support a role for melanization in protection of Langerhans cells or cutaneous immunity. We could detect no relationship between UVB susceptibility and skin color type in our Caucasian

Table IV. Clinical Features and Contact Hypersensitivity Responses of Volunteers with Deeply Tanned Skin

Patient	Sex	Age	Skin	Primary Allergic Reaction	Challenge
66	M	24	T-3[a]	3+	0
67	M	21	T-3	2+	0
68	M	28	T-3	2+	2+
84	M	24	T-3	2+	2+

[a] T, intensity of tan; T-3 is equivalent to B-3 in black-skinned individuals.

subjects [13]. The present results, demonstrating that black individuals with the darkest skin color may be UVB susceptible, further emphasize this point.

Why doesn't melanin in the epidermis protect Langerhans cells or the cutaneous immune system from the effects of UVB radiation? Melanin undoubtedly has a photoprotective role in the skin. Beyond the physicochemical evidence of its ability to absorb light within the UVB spectrum, the fact that black-skinned peoples display little risk for developing squamous/basal cell carcinomas implicates melanin as an important protective agent. In the epidermis of non–sun-exposed skin, melanin granules are found to be localized primarily as "caps" over the nuclei of keratinocytes in the basal layer [26]. As epithelial cells differentiate and "move up" into the stratum spinosum, melanosomes become less obvious. In the strata granulosum and corneum, typical melanosomes are difficult to find. Upon sun (or UVB) exposure, melanosomes disperse from their perinuclear orientation and spread out within the cytoplasm [27–29]. This may have the potential effect of providing a more extensive shield for the cell's own nucleus as well as nuclei of cells (such as Langerhans cells) located adjacent to basal keratinocytes. However, even in heavily pigmented skin, melanosomes are decidedly less prominent as one examines cells higher in the suprabasilar layers of epidermis. It may be for these structural reasons that Langerhans cells are not well protected from UVB radiation, even in melanized epidermis.

The UVB-susceptibility trait, as presently described in humans, also exists in laboratory mice [30]. Our experimental analysis of this trait has revealed that UVB susceptibility is genetically determined in mice by polymorphic alleles at (at least) two independent genetic loci, *Tnf$_a$* and *Lps* [31]. We have recently reported that the inflammatory cytokine, tumor necrosis factor-alpha (TNF_a), can fully mimic the effects of UVB radiation on contact hypersensitivity and is an important mediator of the effects of UVB on the induction of contact hypersensitivity in mice [32]. We suspect that UVB-susceptibility results from excessive intraepidermal production of TNF_a triggered by UVB radiation. The cellular source of TNF_a in this instance has not been identified, although both keratinocytes and Langerhans cells are candidates. The severe disruption of Langerhans cells displayed by the UVB-irradiated epidermis of our black subjects implies that these cells are the targets, rather than the producers, of TNF_a. The fact that Langerhans cells are equally disrupted in UVB-susceptible and UVB-resistant individuals supports this line of reasoning. We suspect, therefore, that the source of TNF_a in UVB-irradiated epidermis may be suprabasilar keratinocytes. Luger et al have amply demonstrated that keratinocytes exposed to UVB light in vitro readily secrete TNF_a [33]. In addition, Oxholm et al [34] have recently reported that TNF_a can be detected in the epidermis of UVB-exposed human skin, and their photomicrographs suggest that TNF_a is in keratinocytes.

From one vantage point, the detection of the UVB-susceptibility trait in black-skinned individuals appears to weaken our claim that this trait is a potential risk factor for skin cancer. The virtual absence of squamous/basal cell cancers in black peoples further undermines our argument. However, these skin cancers are undoubtedly multifactorial in origin, and we would expect that susceptibility to the effects of UVB on induction of contact hypersensitivity would be only one such factor. As mentioned previously, UVB-induced mutations of basal keratinocytes are essential to the etiology of squamous/basal cell cancers. It may be that the superior capacity of heavy melanin deposits within basal keratinocytes to protect these cells from the mutagenic effects of UVB radiation accounts for the resistance of black peoples to skin cancer. In a sense, the increasing incidence of malignant melanomas among young people, even those that are black skinned, and the epidemiologic evidence linking this phenomenon to episodes of severe sunburns lend further support to our contention [35]. Apparently, in the case of severe sun exposure, the heavy melanin deposits in black skin are insufficient to prevent UVB from damaging nuclei of melanocytes and inducing mutations. It is possible that the UVB-susceptibility trait may correlate with susceptibility to malignant melanoma in sunburned black subjects. We intend to test this possibility in the near future.

The frequency of the UVB-susceptibility trait is surprisingly high in both the Caucasian and black individuals we have studied. If this reflects the incidence of the trait in other human populations, an important genetic issue is raised. The UVB-susceptibility trait appears to be deleterious as viewed from the perspective of cutaneous immunity. One does not generally expect deleterious traits to be present in a gene pool at such a high frequency, unless the trait provides an as-yet-unknown advantage. We can only speculate on this important question at this time. UVB radiation has been shown to have effects on the skin beyond the capacity to induce cancers. In laboratory mice, low-dose UVB treatment, similar to that described in this communication, has been shown to interfere with the development of delayed hypersensitivity to the herpes simplex virus [36,37] and *Candida albicans* [38]. Moreover, UVB radiation of skin can dramatically alter the course of cutaneous leishmaniasis in BALB/c mice [39]. In this example, the severe, life-threatening form of cutaneous leishmania infection that is typical of this strain of mice can be suppressed by UVB radiation, thereby promoting survival of the host, albeit without eliminating the chronic infection. It is possible that the genetically determined state of UVB susceptibility to impaired induction of contact hypersensitivity, which is revealed as a deleterious one in our studies, may prove to be an important mechanism for preventing disease mediated by an over-aggressive immune response, and would therefore be retained by selection.

Table V. Induction of Contact Hypersensitivity in UVB-Susceptible Individuals

Patient Number	Challenge Response
44	0
45	3+
69	2+
72	3+
105	2+
81	(+)[a]
82	(+)
101	3+

[a] (+), intense primary allergic reaction after second 2000 μg dose of DNCB; no challenge dose given.

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